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NUTRITION REVIEWS

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AN EPIDEMIOLOGIC STUDY OF HEART DISEASE: THE FRAMINGHAM STUDY

During the period 1948 to 1950 the National Heart Institute established a field study in Framingham, Massachusetts, to investigate the extent and development of cardiovascular disease in a cross-section of the population (T. R. Dawber, G. F. Meadors and F. E. Moore. *Epidemiological Approaches to Heart Disease: The Framingham Study. Am. J. Pub. Health* **41,** *279 (1951)*). With the exception of the abortive attempt of MacKenzie, no community wide epidemiologic study of heart disease had been previously undertaken (Sir James MacKenzie, *The Basis of Vital Activity: Being a Review of Five Years' Work at the St. Andrews Institute for Clin. Res.* London. Faber & Gwyer (1926)). In view of the acknowledged contributions of epidemiologic research to the study of acute illnesses, it was the conviction of a great many workers in public health and preventive medicine that a similar approach to chronic diseases might prove equally fruitful. Because of the scope of such an undertaking it appeared desirable that it be carried out by a Governmental agency.

The study was focused on coronary heart disease and hypertension since these constituted the preponderance of cardiovascular diseases both in morbidity and mortality. An opportunity was also provided to study rheumatic heart disease since, in the detection of coronary heart disease and hypertension, rheumatic heart disease was necessarily identified. Such a study also afforded the opportunity to make limited observations on many non-cardiovascular abnormalities not available in usual clinical studies (J. B. Vander, E. A. Gaston, and Dawber. *N. Engl. J. M.* **251,** *970 (1954)*).

The objective of an epidemiologic study is to characterize the population which develops a disease and to indicate in what particulars it differs from the population not subject to disease. Such a study provides information not obtainable from the usual hospital or clinic study in that the opportunity to study an unbiased control population is automatically provided. The Framingham Study was therefore organized with a view toward describing the characteristics of a population which eventually develops coronary heart disease or hypertension.

The selection of a study population, including the determination of sample size and age distribution, was aimed at providing an adequate group of persons to be observed over 20 or more years and in which there would be a sufficient number of susceptible persons. Later it appeared that a population of about 5,000 persons in the age range of 30 to 59 would yield sufficient numbers of diseased persons for satisfactory analysis. Accordingly, a random sample of the population of Framingham, to include two-thirds of those persons in the desired age range, was selected. The selection of the subjects was random to avoid the bias possible with the self selection which occurs in a study of volunteers.

The task of encouraging the participation of these selected persons was an enormous one and involved the assistance of a large number of Framingham residents whose interest in the study had been kindled. After extended efforts on the part of all concerned, it was possible to bring in for examination 4,469 (68.6 per cent) of the 6,510 selected. Follow-up of the 4,469 subjects has been carried on for six to eight years, and will continue at two year intervals, both by examination in the clinic set-up for the study and from data obtained through other sources.

The major endeavor of an epidemiologic study is the identification and measurement of the various factors which are acting on the subject and which may be playing a

role in the development or progression of disease. For the successful outcome of such a study, it is necessary that there be sufficient variability in the factors studied and that the variability be measurable.

It will be readily apparent that many of the variables which may play a role in the development of cardiovascular disease can be easily determined by simple history or gross observation. Included in this group are such factors as smoking habits, usual hours of sleep, weight gain, baldness, and educational status. Many variables can only be measured inadequately because it is not feasible to make satisfactory observations (*e.g.* a single blood pressure determination may be a poor representation of the 24-hour blood pressure level). Still other factors, although clearly variable, have no method of measurement which can be accepted at the present time (*e.g.* emotional tension).

The task of the cardiovascular epidemiologist is therefore to consider all the factors which may be related to the development or progression of cardiovascular disease, to select those which can be in any way measured, and to make careful observation of these measurable variables. It is hoped that the observations made will be important in that some association will be found between them and the occurrence of disease. If an association is found, the epidemiologist can indicate the direction of further research into the cause and relationship of the association, looking for a clue to the pathogeneses of the disease.

Factors affecting the development of a disease may be related to the host or to the environment. Host factors include blood chemical levels, metabolic level, personality and physical characteristics (*e.g.* baldness, hairiness and obesity).

Examples of environmental factors are those of nutrition, economic status, educational level, occupation and physical energy requirements, stress and "strain of living."

The number of variables affecting each individual is considerable and could not all be determined at one examination. It was therefore necessary to allocate only certain observations to each examination in the hope that over the course of several examinations we would be able to make all the desired measurements. It is hoped that additional factors (*e.g.* personality evaluation, emotional stress, and endocrine make-up) can be studied in future examinations, although up to the present time satisfactory methods for making these observations within the scope of our operation have not been found.

As previously discussed, a vital part of an epidemiologic study is clear identification of diseased persons (*i.e.* case finding). In an acute disease such identification is often clear-cut and easily proven. Chronic diseases are seldom as clearly or definitely defined.

Because of the short period of time between good health and the development of disease in acute illnesses, it is relatively easy to relate any associated factor to the onset of the disease. In a chronic disease, such as coronary heart disease which has an insidious onset and a long subclinical phase which is largely unidentifiable at the present time, it becomes quite difficult to demonstrate the relationship between an observed factor and the development of the disease.

Because such chronic diseases as coronary heart disease and hypertension develop insidiously, it is necessary to follow a large group of people for 20 years or more in order to be able to accumulate enough cases of newly developed disease to make significant observations.

Numerous hypotheses have been proposed relating many factors, both host and environmental, to the development of coronary heart disease or hypertension. Controversy exists regarding practically all such hypotheses. A great deal of controversy has been due to the difficulty of obtaining a population free of bias in which to test the hypothesis. Although most of the hypotheses we can test in the Framingham Study have been tested elsewhere, it is believed that by eliminating in so far as possible, the problem of bias in the population studied, most of

the controversy regarding these hypotheses can be resolved.

Although the relatively small number of persons developing new disease during the period of observation has made the testing of many hypotheses impossible up to the present time, limited observations have already been made regarding a few of the major hypotheses.

It was first possible to make some observations regarding the prevalence of the various types of heart disease in the adult population. Experiences with rheumatic heart disease have been published elsewhere (J. Stokes and Dawber, *N. Engl. J. M.* **255,** *1228 (1956)*). The prevalence of all types of rheumatic heart disease was found to be 2.5 per cent. Observations on the blood pressure findings are in preparation (A. Kagan. "The Range of Blood Pressure in a Normal Population." *In Preparation.*). An estimate of the prevalence of coronary heart disease and the occurrence of new disease during the first four years of the study has been presented (Dawber, Moore, & G. V. Mann, *Am. J. Pub. Health* **47,** *No. 4, part 2, 4 (1957)*). It was found that the prevalence of coronary heart disease in males of ages 45 to 62 was between 4 per cent and 5 per cent and the incidence was approximately 1 per cent per year, again emphasizing the importance of this disease as a contemporary medical problem. It was found that among men of ages 45 to 62 elevation of blood pressure was associated with the development of coronary heart disease. Similarly, fatness was associated, although much of this association could be explained by a high correlation between fatness and hypertension. Hypercholesterolemia was also highly associated with the development of new coronary heart disease in men of this age. Combinations of these three factors produced groups of men with still higher risks of development of coronary heart disease. It was not possible to demonstrate any relationship between tobacco use, or educational status, with the development of new coronary disease.

Future analyses of these same factors will enable better documentation of these associations. In addition many other hypotheses based on observations already made can be tested.

As indicated above it was necessary to postpone observation on certain variables already believed to be related to the development of disease. It is planned to study as many of these as feasible. New hypotheses are suggested from time to time as new information about the disease is uncovered. Where possible these hypotheses will be tested out using the epidemiologic approach.

A number of hypotheses have been proposed linking various factors in the diet to the development of both coronary heart disease and hypertension. Prominent among these hypotheses are excess total caloric intake, high total fat intake, a high intake of animal fat, or high cholesterol intake, all of which are implicated in the production of atherosclerosis. Similarly a high sodium intake has been alleged to cause hypertension (L. K. Dahl and R. A. Love, *J. A. M. A.* **164,** *397 (1957)*). Although many of these hypotheses have already been tested previously, controversy continues to exist because of the population studied and the methods used. It is hoped that the present long term epidemiologic study will resolve some of these differences.

Much of the relationship between diet and atherosclerosis demonstrated in humans has been indirect, equating atherosclerosis with hypercholesterolemia. It still remains for a longitudinal study to follow the dietary of a large group of people until some develop coronary heart disease or other measurable evidences of atherosclerosis in order to be reasonably certain of a more direct relationship.

It is a well recognized, and unfortunately common, finding among workers active in the field of epidemiology involving diet studies that such endeavors are excessively difficult to accomplish in a competent, meaningful way.

In dealing with an adult New England

population a dietary study is a difficult problem. As pointed out previously, in order to study a group for any characteristic it is necessary for the study group to be at least moderately heterogeneous with respect to this characteristic. If everyone were eating about the same foods and in the same amounts food intake would cease to be a variable and measurement of it would be of no value except for comparison of the entire population with some other population. The only recourse is to try to characterize the individual according to the quantities of each food stuff, in the hope that large enough differences will occur.

An almost insurmountable problem in doing diet surveys of large groups is the necessity for a detailed dietary survey by a trained interviewer, preferably a dietitian or nutritionist. The keeping of a list of foods eaten or observations made in the home fall short because of the possibility that the method itself influences the eating habits of those observed, or does not reveal their long-term eating habits.

Many studies utilizing the approach of surveying the buying habits of a community, with respect to a disease, have the objection of failing to relate the dietary characteristics more directly to the individual who develops the disease in order to be sure the association is a straight-forward one. Consequently it becomes necessary to do a dietary survey on each individual involved in the study in order to establish any clear-cut association. The study being carried out in Framingham is ideally suited to such an approach. During the fifth biennial examination at the Framingham Heart Disease Epidemiology Study, an unbiased sub-sample of approximately 1000 subjects was selected. A research type of dietary history based upon the method of Mrs. Bertha S. Burke (*J. Am. Dietet. Assn.* **23,** *1041 (1947)*) was adapted for use in this study in order to obtain an estimate of the average dietary intake over many years, rather than using the 24-hour recall method. Advantage was taken of husband and wife combinations in the sample selected in order to achieve a more complete evaluation of the eating habits of each member. The wife is expected to have more detailed knowledge of the ingredients involved in the preparation of foods. This also allows a check for consistency of diet histories between husband and wife. It is hoped that the information obtained will allow characterization of the study group according to intake of total calories, animal and vegetable fats, animal and vegetable protein and cholesterol. The association of these factors with the present or subsequent development of coronary heart disease will be looked for.

The influence of the large amount of literature being published of late in lay periodicals and newspapers may have affected the eating habits of subjects interviewed to some unknown extent. However, the total fats in the diet still result in a fat intake in excess of 40 per cent of total calories, as noted in the few patients already surveyed in this program.

It is believed that the epidemiologic approach to the study of coronary heart disease and hypertension will point the way to the solution of many of the problems in these diseases. If methods of their prevention and control are to be found, a clear understanding of their epidemiology is imperative. It should be recognized that although the ultimate objective is an understanding of the pathogenesis of these diseases, methods of prevention and control may be instituted long before such knowledge is available, providing their epidemiology is well understood. It may be possible to prevent or control the major causes of heart disease by changing the factors in the host or environment well before their complete pathogenesis is understood.

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URINARY AMINO ACID EXCRETION IN CHILDREN

Interest in amino acid metabolism and excretion has increased with the development of improved and simplified chromatographic techniques in recent years. Many studies, old and recent have measured total amino acids because of the almost insuperable difficulties in quantifying all, or even most, of the individual acids. The advent of microbiological assay techniques did make this possible and has furthered our knowledge considerably. However, the specificity and accuracy of many of these methods are open to question and they are complex and time-consuming.

Chromatography has altered this and it is now relatively easy to get semi-quantitative amino acid "patterns" in insect blood, for example, by paper chromatography, and it is entirely practical to get quantitative data on 20 or more amino acids in somewhat larger samples by column chromatography. Thus, the composition of a wide variety of proteins in body tissues and fluids has been defined in health and disease. The amino acid pattern in serum has been ascertained and some of its variations have been studied.

Although there have been numerous studies of urinary amino acid excretion in the past (*Nutrition Reviews* **6,** *6* (*1948*)), many investigators have used microbiological assays. With increasing interest in the subject, there is a need for defining the normal pattern of amino acid excretion in the urine, as there are wide discrepancies in reported values. Different technical methods, variations due to various factors, fragmentary observations, and lack of uniformity in expressing the results all contribute to the confusion as to normal values.

D. I. Fowler, P. M. Norton, M. W. Cheung, and E. L. Pratt (*Arch. Biochem. Biophys.* **68,** *452* (*1957*)) have published "Observations on the urinary amino acid excretion in man: The influence of age and diet". Amino acid excretion has been variously reported in absolute amounts per day, as a function of age, body weight, surface area, urine volume, or as a fraction of total nitrogen or of creatinine excretion, and the authors point out that there is little parallelism in these. A high value by one standard may be below normal by another, and each has disadvantages. They chose to express urinary amino acid output in terms of creatine excretion since this varies much less with changes in protein intake than does total nitrogen excretion.

The subjects were three premature infants and five full-term infants fed milk formulas, seven infants fed a synthetic formula whose nitrogen was provided by a mixture of 18 L-amino acids, and two ten-year-old children on a mixed diet. The differences in weight and urine volume between children and adults and their variation in disease make the choice of standard a wise one, especially in pediatric work. Fowler and her colleagues used column chromatography and standard methods of chemical analysis. The concentration of ninhydrin-positive components was plotted against an abscissa representing effluent volume from the column. In addition to the recognized amino acid peaks, 13 distinct unidentified peaks appeared. (The available information as to their possible identity is tabulated; the list includes cysteic acid, peptides, sarcosine, citrulline, tyrosine, phenylalanine, β-alanine, β-aminoisobutyric acid and γ-aminobutyric acid.)

An increased excretion of α-amino nitrogen was found in early life, as other workers had reported. By the age of ten years, excretion values were similar to those in adults. In terms of creatine excretion, the premature infant excreted ten times, and the full-term infant three times, that of the ten-year-old. The amino acid excretion pattern differed from that in older children and adults. Four amino acids, taurine, glycine, histidine and methylhistidine, comprised 70 per cent of the total in the latter, 43 to 55 per cent in full-term infants, and 35 per cent in the premature infants. Increased excretion of the following was found in early life:

threonine, serine, alanine, cystine, leucine, tyrosine, phenylalanine, lysine and, especially, glycine and unknown "peak No. 13". A number of amino acids appeared in the urine in early life which were not found in older subjects (proline, hydroxyproline and unknown "peak No. 9"). Taurine was anomalous in that it was present in large quantities in the neonatal period, practically disappeared after that, and reappeared by the tenth year. Some of the abnormalities of prematurity were detected as late as at five months of age.

Variations in protein intake in general caused small changes in urinary amino acid excretion. On the synthetic diet there was increased excretion of several amino acids not attributable to different intakes of the particular acids. The authors inferred that different, and presumably suboptimal, proportions of these compounds had been supplied than existed in natural protein. The total loss was less than 1 per cent of the ingested amino acid. Histidine excretion was decreased on the synthetic diet and unknown "peak No. 9" disappeared, suggesting that the latter was of dietary origin. Work is needed to identify accurately the number of unknown peaks demonstrated in this paper.

The several tables of the experimental results of the authors, together with those of other investigators, should provide a valuable source of comparison for future work on children with various diseases. With accurate normal standards on amino acid excretion available, much of the work on disturbances of amino acid excretion will acquire new value.

ASCORBIC ACID IN MATERNAL NUTRITION

The basic findings of the Vanderbilt cooperative study of maternal and infant nutriton have already been reviewed (*Nutrition Reviews* **12,** *260 (1954)*). Analyses of the interrelationships of biochemical and dietary data on large groups of persons, as represented in this study, are rare. It is enlightening that such detailed reports relative to this large study are being published. Pertinent is the recent report covering data on intakes and serum levels of ascorbic acid in 2,129 pregnant women as related to gestation, season of year, age, parity, height, weight, and other physical findings (M. P. Martin, E. Bridgforth, W. J. McGanity and W. J. Darby, *J. Nutrition* **62,** *201 (1957)*).

Although not high, there was a correlation between recorded ascorbic acid intake, based on a one-week diet record, and the serum ascorbic acid level. For example, in the third trimester in the winter season the median serum ascorbic acid levels were 0.21, 0.36, 0.40 and 0.62 mg. per cent for recorded daily ranges of intakes of below 40, 40 to 79, 80 to 119, and 120 mg. or over, respectively.

There appeared to be a striking decrease in serum ascorbic acid level with increasing parity, which seemed to exceed the expected decline based on recorded ascorbic acid intake. For example, among women of parity 1 and 2, daily recorded intake levels of 56 mg. were associated with serum levels of 0.36 mg. per cent, while in women of parity 5 and over, intake levels of 48 mg. per day were associated with serum levels of 0.24 mg. per cent. The authors suggest that this may reflect the depleting effect of successive pregnancies and lactations.

Gingivitis was a physical finding that appeared to exhibit some relationship to ascorbic acid nutriture. Among women of parity 2 or less with consistently low vitamin C intakes, 19 per cent had gingivitis on at least two examinations. Among the consistently medium and high-intake groups,

the corresponding percentages were 14 and 9, respectively.

An increased frequency of occurrence of premature births was limited to the women with ascorbic acid intakes of 20 mg. per day or less. Although the differences were not as striking, the percentage of premature infants was also higher in the group of women with the lowest serum ascorbic acid levels. The authors point out that these differences were not necessarily indicative of a causal relationship between ascorbic acid and premature births. The data, however, suggest an area for further study. There was no indication that ascorbic acid nutriture was related to the abortions that occurred in 40 of the women in the study.

In general, serum ascorbic acid levels decreased during pregnancy except in women who were on a high intake level. Serum values were further decreased postpartum and were lower for lactating than for non-lactating women. On the average, intakes of 80 to 100 mg. per day supported high levels of ascorbic acid in the serum during pregnancy. Whereas the serum levels averaged 0.7 mg. per cent during the puerperum on intakes (during pregnancy) of 100 mg. or more daily in non-lactating mothers, the serum concentration of lactating mothers did not average over 0.3 mg. per cent on intakes exceeding 120 mg. per day.

In women on low recorded ascorbic acid intakes the mean hemoglobin, packed cell volume, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration, (but not the red cell count), were lower than those in the groups on medium or high intakes. Since low iron intake was associated with low ascorbic acid intake, it was not possible to establish a causal relationship between these blood findings and ascorbic acid nutriture.

Analysis of findings relative to the health of the mother and baby also revealed a possible association between ascorbic acid nutriture and premature separation of the placenta and puerperal fever.

Detailed analysis of data of this type, from the viewpoint of attempting to establish causal relationships between individual nutrients and maternal and infant health, are time consuming and the results are generally difficult to interpret. Such analytical detail, however, is necessary if we are ultimately to have a clear understanding of nutritional interrelationships.

BLOOD AMINO ACIDS, GLUCOSE AND APPETITE

Numerous ideas have been advanced to explain the induction of hunger sensations. One of the early theories, proposed by E. Bulatao and A. J. Carlson (*Am. J. Physiol.* **59,** *107* (*1924*)) was based on the fact that hunger pangs coincided with the waves of contraction of the empty stomach. They also postulated that hypoglycemia was responsible for this effect. W. W. Scott, C. C. Scott and A. B. Luckhardt (*Am. J. Physiol.* **123,** *243* (*1938*)) determined blood sugar on human subjects preceding, during, and after normal hunger periods, and found no variations which indicated that the blood sugar level bore a causal relation to the normal hunger periods of man.

J. Mayer (*New Engl. J. Med.* **249,** *13* (*1953*)) reasoned that it was unlikely that the abundant body stores of protein or fat would decrease much between meals to stimulate the hypothalamic centers. On the other hand, the carbohydrate stores of the body, being small, could easily be depleted. He proposed that hypothalamic "glucoreceptors", sensitive to variations in available blood glucose, were responsible for initiating hunger sensations. Available blood glucose was determined by finding the arteriovenous difference. Since measurement of the ar-

teriovenous blood sugar difference was not possible in the hypothalamic region, the determinations were carried out on peripheral blood with the assumption that the findings were representative of the body generally. Correlation of these findings with hunger periods in subjects on different dietary regimens showed that, on a calorically adequate diet, the arteriovenous (a-v) difference remained high during the day, decreasing only at meal time along with the appearance of hunger feelings. In contrast, hunger appeared earlier on submaintenance diets and the glucose differences become rapidly smaller. However, a recent review on the role of blood sugar and appetite (*Nutrition Reviews* **14,** *332* (*1956*)) urges caution in the general interpretation of peripherally determined a-v sugar differences.

The fact that hunger regulation might be influenced by amino acid metabolism was suggested by C. J. Smyth, A. G. Lasichak and S. Levey (*J. Clin. Invest.* **26,** *439* (*1947*)) who observed that anorexia developed following too rapid infusion of amino acids. This observation was of potential practical importance since the use of protein hydrolysates either intravenously or orally in supplementing a deficient protein intake would be ineffective if it resulted in further decrease of the already flagging appetite of the patient.

S. M. Mellinkoff, M. Frankland, D. Boyle and M. Greipel (*J. Applied Physiol.* **8,** *535* (*1956*)) have recently attempted to correlate the appearance of hunger feelings with variations in serum amino nitrogen and blood sugar concentrations. Hunger was graded on a scale ranging from nauseated to ravenous, and compared with blood sugar and amino nitrogen concentrations determined before and after eating, or before or after infusion of amino acids either alone or with added glucose. During the combined parenteral administration of amino acids and glucose the appetite diminished while the blood concentrations of sugar and amino nitrogen were rising. After the infusion, and while the blood levels of amino acids and sugar were diminishing the appetite was increasing. When amino acids alone were infused the blood glucose dropped to hypoglycemic levels, while the blood amino nitrogen concentration rose and appetite diminished. Similarly, as the blood amino acids decreased the blood sugar increased and appetite returned. It remains to be determined how amino acid levels may be related to appetite in diabetic patients with a high fasting blood glucose and amino nitrogen levels, or in the anorectic patients with hepatitis who may have low amino nitrogen levels after eating.

More recently Mellinkoff, Frankland, and Greipel (*Ibid.* **9,** *85* (*1956*)) have measured the effect of amino acid ingestion and its accompanying hypoglycemia upon the arteriovenous (a-v) blood glucose difference. After the usual night-time fasting period, 16 normal volunteers drank 250 ml. of 10 per cent amino acids. Blood specimens were taken from the antecubital vein and from one finger tip during fasting, 30 minutes after the test meal, and at hourly intervals for a period of four hours. Just before each sample was taken the subject was asked if he were hungry and the responses were graded from minus 1 (nausea) to 0 (not hungry) through plus 4 (ravenous).

For comparison the above procedure was also followed in ten normal volunteers, but a solution of glucose was used instead of amino acids. Calculation of the correlation between changes in the serum amino acid concentration and appetite showed a diminution in appetite as serum amino nitrogen concentration rose, and a rise in appetite as the amino nitrogen concentration decreased. The venous blood sugar and amino nitrogen concentrations changed in opposite directions. When the a-v sugar changes were expressed as the difference between the individual's fasting a-v sugar difference and each subsequent a-v sugar difference, the appetite rose as the a-v sugar difference

diminished and vice-versa. Following the ingestion of glucose there was a significant correlation between changes in appetite and changes in both the venous blood sugar and the a-v sugar difference. There was no correlation between changes in appetite and changes in amino nitrogen concentration following the ingestion of glucose. Mellinkoff, Frankland and Greipel, however, do not claim that these experiments prove or disprove the a-v sugar hypothesis, but rather that the data indicate metabolic changes associated with appetite variations under specific circumstances. One mechanism for the depression of venous blood sugar after ingestion of amino acids is the possible uptake of glucose by the muscles, suggested by the fact that the venous blood sugar was generally lowest when the a-v sugar difference was greatest.

Further experiments are necessary to determine the mechanism by which amino acid ingestion depresses blood sugar concentration and appetite, and the role of the a-v sugar difference under these circumstances. For the present, the exact mechanism by which appetite is stimulated remains shrouded in mystery.

STUDIES ON POSTGASTRECTOMY STEATORRHEA

W. W. Shingleton *et al.* (*Surgery* **42,** *12* (*1957*)) have conducted studies designed to clarify the processes involved in the steatorrhea which develops in approximately one half of patients following gastrectomy.

Earlier studies by the same authors, using I^{131}-labeled triolein, had shown that approximately one half of the patients had significantly low blood radioactivity and abnormally high fecal radioactivity following the test meal. Previous studies (*Nutrition Reviews* **14,** *266* (*1956*)) had demonstrated a high fecal fat excretion in postgastrectomy patients.

The present study, however, was conducted with the use of both radioactive neutral fat (triolein) and a fatty acid (oleic acid) in order to evaluate both the digestive function and the absorption in these patients. The effects of the site of anastomosis after gastrectomy, of the caloric intake, and of the addition of bile and a pancreatic preparation to the test meal were also evaluated.

The test meal was given after a four-hour fast and contained 25 microcuries of I^{131}-labeled triolein or oleic acid in 0.5 cc. of the same carrier, and 20 drops of Lugol's iodine; 3 gelatin capsules containing barium sulfate were given in addition. After the second hour following the meal, hourly blood specimens were drawn until the sixth hour. Fluoroscopic localization of the test meal was done at the third hour, and all feces were collected for 48 hours. Twenty-four normal control patients and 14 postgastrectomy patients were studied in this manner.

All had lower blood radioactivity and elevated fecal radioactivity following the triolein test meal. With the oleic acid test meal, only three patients had blood values slightly below normal, only three had fecal values slightly above normal, and only one had a significantly abnormal fecal radioactivity.

In terms of the type of anastomosis, the blood and fecal values following the triolein test meal were normal, or only slightly abnormal, in patients who had had a Bilroth I procedure, and were significantly more abnormal in the group of patients who had undergone a Bilroth II procedure. On the other hand Robins *et al.* (*Surgery* **41,** *248* (*1957*)) during the course of metabolic studies, failed to demonstrate any significant difference in fecal fat and nitrogen excretion when comparing patients with a Bilroth I and patients with a Bilroth II type of gastrectomy. Simultaneous fecal fat determinations and radioactive tracer studies carried out on such a group of patients would prob-

ably help disclose the reason for this discrepancy.

The caloric intake was not statistically different in patients who were above their ideal weight, as compared with those who were below it.

Neither the pancreatic preparation nor bile salts, when added alone to the test meal, produced any significant change in the blood or fecal radioactivity. Intravenous administration of secretin similarly failed to improve the results of the test meal.

However, when the pancreatic preparation and bile salts were both given with the triolein test meal, there was a prompt and marked increase in blood values of radioactivity.

The results of this study suggest, therefore, that the primary disturbance in postgastrectomy patients with steatorrhea is more likely to be a deficiency of digestion than an error in the absorption of fat. The use of a combination of a pancreatic preparation and bile salts is suggested as a means to improve this digestive disturbance.

THE PYRIDOXINE REQUIREMENTS OF INFANTS

The experimental production of vitamin B_6 deficiency in man (*Nutrition Reviews* **12,** *10 (1954)*), and considerable animal experimentation have suggested that convulsions might well be a critical manifestation of this deficiency in infancy. During 1953 the occurrence of hyperirritability and convulsions in approximately three out of every 1000 infants fed a proprietary formula approximating human milk in composition was found to be associated with a decreased content of vitamin B_6 in this preparation, which had been brought about by a change in the method of heat sterilization used (*Ibid*, **13,** *342 (1955)*). The hyperirritability and convulsions in the 300 infants showing these symptoms disappeared following pyridoxine administration, or change to a diet more adequate in pyridoxine. The supplementation of the proprietary formula with pyridoxine was followed by the disappearance of this clinical syndrome (D. B. Coursin, *J. Am. Med. Assn.* **154,** *406 (1954)*; C. J. Molony and A. H. Parmelee, *Ibid.* **154,** *405 (1954)*; *Vitamin B_6 in Human Nutrition—Report of the 10th M. and R. Pediatric Research Conference, Columbus, Ohio, M. and R. Laboratories (1954)*).

These observations stimulated several studies of the vitamin B_6 requirements of infants. Coursin (*Am. J. Dis. Child.* **90,** *344 (1955)*) pointed out that the infants receiving the milk formula referred to above (containing about 0.06 mg. of vitamin B_6 per liter) were between 3 and 6 months of age. This nearly complete formula probably accounted for the absence of obvious changes in growth, red cell formation, or apparent immunity. The most obvious clinical findings were the repeated seizures of short duration which were most noticeable on stimulation from movement, feeding, and sound. Electroencephalograms were abnormal only during the seizures. Six infants in the group studied by Coursin showed lower thresholds of reaction to controlled auditory stimuli during electroencephalography. These changes were interpreted to represent definite deviation from normal thresholds, and this increased excitability was shown to be readily corrected by the addition of 0.2 mg. of pyridoxine hydrochloride per liter of formula.

Since only 300 cases with severe irritability were found in a national survey, Coursin suggests that some selective conditions must prevail in order to give rise to clinical symptoms. A relative deficiency at birth or lowered thresholds of reaction due to intracellular changes in the metabolism of vitamin B_6 are suggested as possible mecha-

nisms. Seizures due to vitamin B_6 deficiency could be relieved by changing the infant's diet to simple lactose-water. The addition of protein to the diet caused a reappearance of irritability and convulsions. These again disappeared by increasing the dietary intake of vitamin B_6 by 200 micrograms per liter. These data suggest that the B_6 requirement is related to the protein intake. Coursin concludes that a dietary intake of 60 micrograms per liter is suboptimal with the production of lowered thresholds of central nervous system activity in a significant number of infants.

A first approximation of the requirements of infants for pyridoxine has been made by O. A. Bessey, D. J. D. Adam and H. A. Hansen (*Pediatrics* **20,** *33* (*1957*)). In five infants fed the proprietary formula referred to above, and having a vitamin B_6 intake of .085 mg. per day, convulsions ensued which were eliminated by the administration of a diet of evaporated milk providing .26 mg. per day of pyridoxine. Xanthurenic acid excretion was increased in the only one of these infants thoroughly studied, and it was found that 1.0 to 1.2 mg. per day of vitamin B_6 were necessary to prevent this increased excretion of xanthurenic acid. These authors also studied two infants, aged 4 and 12 months, who had received only human milk. Analysis of the milk from one of the mothers showed it to supply 0.067 mg. of vitamin B_6 per day. These two infants both responded to treatment with an evaporated milk formula supplying 0.26 mg. of vitamin B_6 per day. Xanthurenic acid excretion was also increased and the amount of vitamin B_6 required to prevent it was in the range of 1.0 to 1.4 mg. per day.

In contrast to these observations, which are similar to those reported by Coursin and others, two infants having convulsions beginning in the neonatal age group were studied at the ages of 8 and 14 months. At that time they were receiving evaporated milk and pureed foods supplying approximately 0.3 to 0.4 mg. vitamin B_6 daily. Increased xanthurenic acid excretion following a tryptophan load was not present in one of these infants, and the elevated excretion was minimal and responded to approximately 0.4 mg. per day of vitamin B_6 in the second. Control of convulsions in these two infants necessitated large doses of pyridoxine, 2 mg. daily in one case and 5 mg. daily in the other. Bessey, Adam, and Hansen suggest that these two patients differ from those developing seizures on diets which appear to be deficient in vitamin B_6, and that their convulsions were related to an aberration in the metabolism of this vitamin, since relatively small amounts of vitamin B_6 were necessary to eliminate the excretion of xanthurenic acid while much larger amounts were necessary to eliminate the convulsions.

These last two patients appear to have a condition very similar to that of pyridoxine dependency, described by A. D. Hunt, Jr., J. Stokes, Jr., W. W. McCrory, and H. H. Stroud (*Ibid.* **13,** *140* (*1954*)). The mother of their patient had been treated with large amounts of pyridoxine parenterally during her pregnancy and these authors suggested that this treatment might in some way have influenced the development of pyridoxine dependency in their patient. She had seizures within 48 hours when she was not receiving 2 mg. of pyridoxine hydrochloride daily. Xanthurenic acid excretion following a tryptophan load was not increased in this patient and the electroencephalogram was abnormal only during the seizure episodes. The cases of Bessey, Adam and Hansen were not born to mothers who had received massive doses of pyridoxine during their pregnancies. The discrepancy between the amount of pyridoxine required to suppress abnormal xanthurenic acid excretion and to eliminate the convulsions in these infants as compared to those ingesting diets deficient in vitamin B_6 certainly does suggest that aberrant metabolism rather than dietary deficiency is the most likely cause of this syndrome.

Bessey, Adam and Hansen studied the

xanthurenic acid excretion following tryptophan administration (0.54 mg. per kg.) in ten control infants receiving diets containing more than 0.20 mg. of vitamin B_6 per day. There was no increased excretion of xanthurenic acid in this group. Two of these infants, who were placed on the formula deficient in vitamin B_6 for one month, excreted amounts of xanthurenic acid following the load test equivalent to the excretion in four infants who had developed the defect spontaneously. The ingestion of 0.3 to 0.4 mg. of pyridoxine per day, an amount less than one-third found necessary to suppress xanthurenic acid excretion in the infants initially seen with convulsions, suppressed xanthurenic acid excretion. These findings are interpreted to suggest that the requirement for vitamin B_6 of the seven infants who had developed the clinical manifestations of the deficiency was, for some reason, greater than that of the average infant.

It may be concluded from these studies that intakes of less than 0.1 mg. of vitamin B_6 per day are associated with the development of clinical manifestations in a significant number of infants. Intakes of 0.3 mg. per day or more have not been associated with these symptoms nor with increased excretion of xanthurenic acid following a tryptophan load test in normal infants. The occurrence of convulsions responding to vitamin B_6 administration in infants ingesting diets apparently adequate by the above criteria suggests that a metabolic abnormality in pyridoxine metabolism may occur. In two of the three infants with this condition so far reported, an intake of at least 2 mg. per day was necessary to suppress the convulsions. The administration of 5 to 10 mg. of pyridoxine hydrochloride daily to any infant with convulsions resulting from inapparent cause might bring to light further instances of this unusual anomaly.

THE CAMEL AND WATER

From time immemorial the beast of burden in the larger arid areas of the world has been the camel, which has been recognized both in lore and legend as being able to do without water for long periods of time. Surprisingly enough, there has been very little exact information as to the degree to which, and the mechanisms whereby, the camel is physiologically adapted for its work in desert areas.

The study by B. Schmidt-Nielsen, K. Schmidt-Nielsen, T. R. Houpt and S. A. Jarnum (*Am. J. Physiol.* **185,** *185* (*1956*)) provides some of the information on the physiological adaptation of the camel to drought conditions. They established a laboratory in the oasis Beni Abbes in the Sahara Desert of southern Algeria. Here they checked on the length of time 12 camels were without water prior to slaughtering. Some of these animals were reported to have been without drinking water for periods as long as 60 days. In spite of the meager water intake, the tissues from these camels appeared to be in a normal state of hydration. These animals were killed in the winter and spring when camels are able to secure their water entirely from the herbage growing in the desert and along the dry river beds.

The above workers studied in some detail a young female camel about three and one-half years old (increased in weight from 223 kg. to 273 over the eight month period) and an uncastrated adult male about seven years old (weight, 479 kg.). An adult male donkey about five years of age was also studied (weight, 100 kg.). These animals were kept outside the laboratory during the day so that intake and excretion of water could be measured. The water excretion could be studied fairly accurately since the camel prefers to urinate standing up. By tying the animal down with a rope around the "knees," the animal could be prevented

from standing for periods up to eight hours. Within half an hour after the camel was permitted to stand, it urinated.

One of the camels was kept in the direct summer sun for 17 days (maximum daytime temperature averaged 38°C.). During this time, it ate dry dates and hay. Even though it lost 37 per cent of its initial body weight during the period of dehydration, it recovered without any ill effects. This is a truly phenomenal ability. Under similar circumstances, the donkey would have become severely dehydrated and would have died after four days of water deprivation. A man would have died on the second day.

The preceding experiment and other observations showed that camels maintained their appetite as long as their water loss represented something less than 20 per cent of their body weight. As weight loss progressed, they gradually lost their appetites. The ability of the camel to continue eating in the face of such drastic dehydration is in marked contrast to man and other animals who soon lose their appetites when deprived of water.

Water balance studies showed that the camel loses water at a lower rate than the donkey. In the winter when the camels get nothing but the dry herbage growing in the desert and along the dry river beds, the rate of water loss amounts to less than 1 per cent of the camel's body weight per day. The donkey, on the other hand, under similar circumstances, loses approximately 2 per cent of his body weight.

Even in the summer when the maximum temperature reaches 40°C., the camel, during periods of water deprivation, loses water amounting to less than 2 per cent of his body weight per day. The donkey under similar circumstances loses water at the rate of 7 per cent of his body weight. If unlimited amounts of water were available, both the camel and the donkey lost water at higher rates (4 and 9 per cent, respectively).

Surprisingly enough, the fur on the camel is an effective means for reducing the rate of water loss. The camel that had been sheared showed a water loss approximately 1½ to 2 times that of the unsheared animal. Shearing the second animal brought its rate of water usage up to that of the preceding one.

The loss of water by the camel via the urine during periods of water restriction averaged 0.75 liters per day. When water was severely restricted, the urine volume averaged 0.5 liters per day. During periods of unrestricted water intake, urine volumes as high as 7 liters per day were seen.

The donkey under similar circumstances had a urine volume that was approximately three times that of the camel when both sets of figures were calculated as percentages of body weight.

The camel and the donkey have phenomenal abilities to consume water. After water deprivation, producing body weight loss of less than 20 per cent, the camel could consume enough water to restore the lost body weight within a period of less than ten minutes. In some experiments, the water consumed within the ten minute period amounted to 66 kg. The donkey was able to consume 10 liters of water within 70 to 80 seconds.

A partial explanation for the low water requirement of the camel, when exposed to hot desert conditions, is found in the increase in his body temperature which occurs under these conditions. K. Schmidt-Nielsen, B. Schmidt-Nielsen, Jarnum, and Houpt (*Ibid.* **188,** *103* (*1957*)) found that during periods of water restriction in the summer, the camels' rectal temperatures showed variations ranging from a low of 34.2° to a high of 40.7°C. These temperature variations usually occurred with the lowest reading at 8 in the morning and the highest at about 7 in the evening. Under similar circumstances, the temperature fluctuations in a normal man are likely to be less than 2 degrees. During periods of plentiful water supply, the body temperature fluctuations in the camel ranged from 2 to 3 degrees each day.

The increase in the body temperature

during the hot day represents the accumulation of a considerable amount of heat which is dissipated during the cooler part of the night. The camel's ability to store heat permits a marked reduction in his requirement for water, which man requires for evaporative cooling purposes. The donkey is between man and the camel in being able to store heat when the gain thereof exceeds the loss.

These studies provide us with quantitative data which help to explain the unique adaptative ability of the camel to water deprivation. They also raise questions. Does the camel show a change primarily in its extracellular fluid space during periods of severe water deprivation? What are the physiological mechanisms whereby he is rehydrated after consuming as much as 66 liters of water within a period of ten minutes? Again, the importance of water as a nutrient is emphasized.

BLOOD VOLUME, BODY WATER AND FAT IN THE HORSE

Thoroughbred horses have more erythrocytes, a higher percentage of eosinophils, and a greater concentration of hemoglobin than Percherons, according to B. F. Trum (*Am. J. Vet. Res.* **13,** *514 (1952)*). In order to interpret these hematological differences accurately in terms of the total numbers of red cells or eosinophils available to the organism, L. M. Julian, J. H. Lawrence, N. I. Berlin and G. M. Hyde (*J. Applied Physiol.* **8,** *651 (1956)*) believed it was necessary to rule out dilution or concentration of the plasma by making precise determinations of the plasma volume. Only a few reports may be found in the literature dealing with the blood volume of horses, and no information as to the breed of animal was included. Furthermore, previous determinations employed infusion of saline or dyes and may be considered approximate in view of comparisons with the more reliable isotope dilution techniques. The failure to recognize the importance of allowing for body fat when interpreting blood volume values, as percentage of body weight, has further decreased the reliability of the earlier studies. Accordingly, determinations of body water and blood volume were made on ten mature horses.

There were four Percherons, two Thoroughbreds, two registered Quarter horses, one Arabian stallion and one American Saddle bred. Red cells labeled *in vitro* with P^{32} were used for the determination of blood volume. Tritiated water was injected to determine total body water. One external jugular served for injection, the other for sampling after homogeneity had been achieved. Employing the assumption that lean tissues average about 73 per cent water and fat tissue has no water, the body fat was calculated from body water and body weight using the following formula: per cent body water = 0.73 (100 − per cent fat). The average blood volume of "hot-blooded" horses (Thoroughbred, Quarter, Arabian, and American Saddle bred breeding) was 109.6 ml. per kilogram of body weight compared with 71.7 ml. per kilogram of body weight for Percherons.

Calculation of blood volumes as ml. per kilogram of fat-free body weight permitted a more accurate evaluation of the differences between the two groups. The "hot-blooded" horses averaged 126.0 ml. per kilogram of fat-free body weight while the "cold-blooded" horses averaged 94.9 ml. per kilogram of fat-free body weight. The fact that the body water content of the "hot-blooded" horses was 63.8 per cent of body weight compared with 55.2 per cent of body weight for the Percherons indicated that the latter group had more fat on the average (24.5 per cent of body weight) than the "hot-blooded" horse (12.8 per cent of body weight). These differences appear to be clear-cut in spite of some overlapping between the groups. The finding that "hot-blooded" horses have

higher hematocrits than Percherons, coupled with a greater volume of blood per unit of body weight, means there is an even greater volume of red cells available to the tissues of the "hot-blooded" horse.

It is conceivable that the development of horses for speed and endurance, for speed only, or for power without speed by selective breeding could result in the intensification of other physiological characteristics related to these blood volume changes. For example, the rate of blood flow and the respiratory volume and rate may vary greatly in these different breeds.

LYSINE REQUIREMENTS OF THE YOUNG PIG

A number of studies in the past few years have added considerably to our knowledge of the requirement of the pig for individual amino acids (*Nutrition Reviews* **9,** *220 (1951)*; **12,** *68 (1954)*; **13,** *304 (1955)*; **14,** *50, 301 (1956)*). Recent studies on the lysine requirement of the pig of suckling age (two to six weeks) and of the weanling pig (beyond six to eight weeks) are now available.

H. D. Hutchinson *et al.* (*J. Animal Sci.* **16,** *553 (1957)*) have studied the requirement of the suckling pig. The basal ration, low in lysine, was fed *ad libitum* and consisted of dry skim milk, sesame oil meal, glucose, and corn starch with smaller amounts of dried whey, fat, antibiotics, and ample mineral and vitamin supplements. The lysine content of this basal ration was found to be 0.525 per cent as determined by microbiological assay. The protein content of the ration was 14.25 per cent. Graded levels of DL-lysine, from 0 to 1.0 per cent, were added to the ration for a total of six groups. Sixty pigs were used, two weeks of age and averaging 9.5 pounds each, in two experiments.

The results showed that the addition of DL-lysine to the basal ration significantly increased the rate of gain and feed efficiency over a four week period, as was expected. Maximum effects were obtained in both experiments when a total of 0.925 per cent of L-lysine activity was present in the ration (the authors assumed that the D isomer of lysine was not active for the pig). When this level of lysine is expressed as a fraction of the protein it amounts to 6.6 per cent.

The authors did not study the effect of different levels of protein on the lysine requirement nor did they use a control group fed a stock ration to see whether or not growth on the lysine supplemented diet was optional. The latter would have been important since their pigs weighed 24.5 and 20.3 pounds at six weeks of age in the two experiments, respectively. These figures are somewhat low.

In a second paper Hutchinson, Jensen, Terrill, and Becker (*Ibid.* **16,** *558 (1957)*) studied the lysine requirement of the weanling pig. A total of 36 pigs was used weighing approximately 35 pounds each (age of the pigs not given). Only one experiment was reported and the pigs were kept on the experiment for only three weeks. They were divided into groups of six pigs each and given graded levels of L-lysine from 0 to 0.50 per cent. The basal ration, similar to that used in the previous study, contained 0.32 per cent of lysine and 11.7 per cent of protein. The pigs were given access to individual feeding crates twice daily and were "fed all they would consume at each of these two feedings". (The authors do not make clear how long the pigs were fed or how six groups of pigs were fed in only three pens containing 12 pigs each when only six pigs were used in each lot, but presumably some means was used to separate the groups when feeding crates were used.) Water was available at all times.

Pigs fed the ration containing a total of 0.52 per cent L-lysine made significantly faster gains than those fed lower levels (0.32 or 0.42 per cent). Three different levels of lysine higher than 0.52 per cent (up to

0.82 per cent) did not result in faster growth or improved feed efficiency. Pigs receiving the optimal level of lysine, 0.52 per cent or 4.5 per cent of the dietary protein, gained an average of 1.04 pounds per day over the three week period and required 2.67 pounds of feed per pound of gain.

Several weaknesses may be pointed out in the experimental procedure in this second paper, The most obvious is that the results are based solely on one experiment. The length of time of the experiment (three weeks) seems rather short and the daily gain, 1.02 pounds, is not as high as often seen. Again, results with a stock ration known to be adequate would have been interesting. Also, it would be important to know if the results would have been the same if *ad libitum* feeding had been used rather than feeding twice a day. It should be pointed out that the lysine requirement of the pig, as suggested by the National Research Council (*Nutrient Requirement for Swine, National Academy of Sciences—National Research Council Publication 295, 1953*) is 1 per cent of the diet. However, this paper and others (*Nutrition Reviews* **13,** *304 (1955)*; W. H. Pfander and L. F. Tribble, *J. Animal Sci.* **14,** *345 (1955)*) indicate that the requirement may be considerably less than 1 per cent.

The results contribute preliminary information on the lysine requirement of pigs. As is true with other amino acids, the lysine requirement of the pig of suckling age appears to be larger than that of the weanling pig when calculated on the basis of per cent of total ration. Actually, proof of this statement must await additional experiments in which the two age groups are compared under identical conditions (with rations of varying protein contents and with the same source of lysine).

Experiments on this subject are useful to the nutritionist in comparing the lysine requirement of different animals for estimations of the requirements of children (*Nutrition Reviews* **14,** *101 (1956)*). Lysine is known to be one of the more important amino acids in human nutrition. Interest in this amino acid has been generated by potentially unlimited supplies from chemical synthesis. Although the experiments reported here are useful, it is hoped that future animal studies with lysine will be made under better experimental conditions.

PROVITAMIN D IN ANIMAL TISSUES

Susceptibility to rickets seems to vary from species to species, and it cannot be stated with confidence that availability of vitamin D (whether ingested as such or formed photochemically *in vivo*) is a decisive factor in the etiology of this disease. Even if considerable circumstantial evidence were brought to bear on the subject, there would yet be uncertainty; the limiting factor could be the amount of provitamin D_3 (7-dehydrocholesterol) absorbed or formed *in vivo*, or it could be the accessibility to ultraviolet light of the provitamin in the body.

H. R. Rosenberg (*Vitamins, Interscience, N. Y., p. 347 (1942)*) found no provitamin D in the preen glands of chickens but quite substantial amounts in the skin of the feet. However, in the human, provitamin D does not seem to be present to any considerable extent in the sebum. Climatic or seasonal factors might be limiting with respect to the available light energy of wave lengths less than 300 mμ.

With respect to fur- or wool-bearing animals, it is difficult to see how ultraviolet light could penetrate fur or wool sufficiently to act upon the provitamin D of skin. According to G. N. Festenstein and R. A. Morton (*Biochem. J.* **60,** *22 (1955)*) the whole problem is seen in its most acute form in the case of the herbivorous animal, which apparently has no intake of preformed vita-

min D unless it is by licking irradiated lipids from wool or fur. Sheep are not known to lick themselves or other sheep and yet it seems clear that this species, and some others which seem least likely to ingest or produce vitamin D, are not particularly susceptible to rickets.

The demonstration by M. Glover, J. Glover, and R. A. Morton (*Biochem. J.* **51,** *1 (1952)*) that reversible formation of 7-dehydrocholesterol from cholesterol can occur in the intestinal wall of the guinea pig affords a valuable clue to possible origin of provitamin D in many species. The proof that dehydrogenation can occur does not, however, mean that provitamin D is always endogenous in origin; different species may differ with respect to their efficiency in carrying out the conversion or in their capacity to absorb related sterols such as ergosterol.

Many questions thus remain to be answered. As a preliminary study of these factors, G. N. Festenstein and R. A. Morton (*loc. cit.*) have studied the occurrence of provitamin D in various animal tissues. These included frog ovaries, herring roe and milt, pig intestine and skin, horse stomach and intestine, ox intestine, and sheep intestine.

Provitamin D (7-dehydrocholesterol) was extracted with ether following preliminary drying of tissue by grinding in the presence of anhydrous disodium sulfate. Concentrations of the provitamin were determined spectroscopically and by chromatography (grade O alumina).

The total dried ovaries of prehibernation frogs (*Rana temporavia*) showed 0.036 per cent of dehydrosteroid. Prespawning frog ovaries showed 0.011 per cent of this substance. Herring roe and milt contained only small amounts of substances showing ultraviolet absorption bands of 7-dehydrosterol. Pigskin was found to contain 0.002 per cent 7-dehydrosterol. The sterol from pig intestine also contained small quantities of materials with ultraviolet absorption maxima at 253, 265 and 272 millimicrons and traces of 7-dehydrosterol. Horse intestine yields crude cholesterol containing 0.014–0.042 per cent of 7-dehydrosterol. Most of the provitamin was present in the first 10 feet of the intestine. The cholesterol fraction from stomach outer layers contained approximately 0.0010 per cent of the provitamin. The cholesterol from ox intestines contained 0.011 per cent and that from sheep intestine contained 0.037 per cent of 7-dehydrosterol.

The authors are of the opinion that the distribution of materials showing the characteristic absorption spectrum of steroid -5:7-dienes (*e.g.*, ergosterol and 7-dehydrocholesterol) is consistent with the widespread occurrence of a special dehydrogenase, the normal substrate of which is cholesterol. Although the 7-dehydrosterol content of the intestines is low in ox, pig, horse, and sheep, the occurrence of the enzyme is still a probability. The importance of its role in the production of vitamin D is not clear at the present stage of investigation.

VITAMIN A IN INTERMEDIARY METABOLISM

Vitamin A was one of the first vitamins to be discovered. Its profound effects on growth and development, and on the maintenance of normal vision in the rat, indicated early in nutrition studies that this was a vital dietary substance. Yet, after forty years, the role of this vitamin in metabolism has been solved only partially. Its participation in the sequence of reactions in the cycle of dark vision has been clearly worked out (*see Nutrition Reviews* **7,** *339 (1949)*; **8,** *198 (1950)*; **10,** *300 (1952)*).

In sharp contrast to the latter, no specific function for vitamin A at the level of inter-

mediary metabolism, which would relate to its more general effects on growth, has been forthcoming. It is known that vitamin A is involved in the maintenance of normal, stratified epithelial tissue (*Ibid.* **11,** *244 (1953)*). Despite intensive study by many investigators, neither this association nor others that have been postulated have been carried to the enzyme level of metabolism.

It is of interest, therefore, that a recent report by Wolf, Lane and Johnson (*J. Biol. Chem.* **225,** *995 (1957)*) should implicate this fat-soluble vitamin in enzymatic reactions concerned with carbohydrate metabolism.

Weanling male rats were fed a vitamin A deficient, purified diet. Two types of control animals, given vitamin A, were used, one fed *ad libitum*, the other pair-fed to the deficient rats. In each experiment, only one rat of each type was used. When the rats on the deficient diet developed anorexia, weight loss, hair roughness and xerophthalmia (after four to five weeks), all animals were subjected to isotope experimentation.

The general plan was to administer C^{14}-labeled compounds intraperitoneally and to kill the animals after six hours, analyzing various tissues for the C^{14} content of specific metabolites.

The distribution of the C^{14} from the first labeled compound, acetate-1-C^{14}, was determined in the expired CO_2, the combined liver and brain cholesterol, the combined liver and brain fatty acids, and in the liver protein, aspartic acid and alanine.

No difference was found in the specific activity of the expired carbon dioxide from the three types of animals. The specific activity of cholesterol isolated from the deficient rat was three times that of the pair-fed control animal, and six times that of the *ad libitum* fed rat. The authors considered these differences insignificant and interpreted this as indicating that vitamin A is not required for cholesterol biosynthesis. Also, when the isolated fatty acids were tested, those from the deficient animals had more specific activity than did those from two types of controls, and this was interpreted as evidence for the nonessentiality of vitamin A in fatty acid synthesis. The authors do not suggest, as would seem logical, that vitamin A may have a regulatory function in the formation of cholesterol and fatty acids.

These results indicated that vitamin A plays no important role in the metabolism of fats. Therefore the investigators next examined various aspects of carbohydrate metabolism. Since the vitamin A deficient animals had no liver glycogen it was necessary to inject normal glucose prior to giving the labeled acetate so that glycogen could be isolated. When 600 mg. of glucose was injected intraperitoneally, pair-fed rats, after four and a half hours, had one and one-half to three times as much liver glycogen as did the deficient rats, while *ad libitum* fed rats had three to seven times as much. When the labeled acetate was given 30 minutes following the glucose injection with the glycogen isolated four hours later, the specific activity of C^{14} in the pair-fed rat was eight times that of the vitamin A deficient rat. This result was interpreted as showing that vitamin A deficiency has a pronounced effect on the reversal of glycolysis. (For some reason, not mentioned, the *ad libitum* fed rat had essentially no radioactivity in its liver glycogen).

Analysis of liver protein, and of alanine and aspartic acid isolated from hydrolyzed liver protein, revealed no significant differences in specific activity in these substances from the three types of rats. It was concluded that no impairment in the tricarboxylic acid cycle occurred in vitamin A deficiency. Furthermore, it appeared to the authors that the impairment in reversal of glycolysis, noted above, must lie in some reaction between pyruvate and glucose.

Results obtained by the administration of glucose-1-C^{14} to rats showed that the interference in glycolysis reversal did not lie in the final conversion of glucose into glycogen.

To verify the defect in glycogenesis in the vitamin A deficient rat, lactate-1-C^{14} was tested. This compound would have to be oxidized to pyruvate and then taken through the series of reactions to glucose and, finally, glycogen. Again, the isolated glycogen from the deficient rat had considerably less specific activity than that from the pair-fed normal animal; the *ad libitum* fed rat had a lower specific activity than the deficient animal. This latter discrepancy was not explained.

In an attempt to pin-point more closely the enzymatic reaction which was involved, glycerol-1,3-C^{14} was tested. When the liver glycogen was analyzed, that from the pair-fed rat had a specific activity 17 times that of the deficient rat. An *ad libitum* fed rat was not used in this experiment. For some unexplained reason, the amount of glycogen isolated from the control rat in this experiment was only 86 percent of that of the deficient rat, whereas in all other experiments the deficient rat deposited considerably less glycogen than the control rat. From these results, the authors concluded that the defect in glycogenesis in the vitamin A deficient rat lies in the reactions by which glucose is formed from triose.

This paper is open to a number of criticisms. For example, the use of single animals in each experiment is questionable. The inconsistent results with *ad libitum* fed rats, noted above, leaves the reader in somewhat of a quandary. Moreover, the validity of the entire study rests on the physiological condition of the vitamin A deficient rats. From the brief description given, it would appear that the rats were used for experiment when they were in the advanced stage of deficiency bordering on morbidity. It has been shown in numerous studies (*see T. Moore, Vitamin A, p. 295, Elsevier Publishing Co., New York (1957)*) that such animals have a wide variety of physiological derangements, most of which are probably unrelated at the biochemical level to the specific vitamin in question. Consequently, the implication of this paper that vitamin A is closely associated with the incorporation of acetate into glycogen would appear unfounded.

This criticism is borne out by a subsequent report by the same group (G. Wolf, S. R. Wagle and B. C. Johnson, *Fed. Proc.* **16**, *272 (1957)*). In pursuing this problem further, they found that the injection of cortisone into vitamin A deficient rats for four days prior to injecting labeled acetate completely eliminated the differences in specific activity of the glycogen previously noted. Thus, the effect of vitamin A deficiency appears to be indirect and probably exerted through the adrenal hormones. Possibly the stress of the deficiency state initiated the events observed.

These studies point up the difficulties of attempting to show the specific participation of fat soluble vitamins in enzyme reactions. Although many metabolic defects have been noted in deficiencies of vitamins E, D and K as well as A, no definite role for any of these vitamins as a coenzyme, or as an enzyme activator or inhibitor, is definitely known. The studies of Wolf and associates re-emphasize this fact. It is to be hoped that, with a proper appreciation of the problems involved, biochemists will intensify their efforts in this field, one which they have long tended to avoid.

A GOITROGENIC FACTOR FROM THE GROUNDNUT

Two papers by V. Srinivasan, N. R. Moudgal and P. S. Sarma (*J. Nutrition* **61**, *87, 97 (1957)*) provide concrete evidence for the existence of a goitrogenic agent in the groundnut (peanut, *Arachis hypogaea*). Their study represented an extension of earlier work by a number of investigators who, as a result of reports that goiter may occur under

conditions of normal dietary intake of iodine, investigated the goitrogenic properties of a number of species of plants (*Nutrition Reviews* **8,** *196 (1950)*). The occurrence of goitrogens in various foodstuffs, particularly in members of the Brassica (cabbage) family, was clearly established.

Srinivasan and associates studied the effect of groundnut supplements on the growth of young albino rats using a basal ration consisting of wheat gluten, 30 per cent, and ground yellow corn, 68 per cent. The remainder of the diet consisted of an iodine-free salt mixture and a vitamin mixture. The various experimental groups received different groundnut fractions added at the expense of the entire ration. Thyroid size, iodine content of the thyroid, uptake of radioactive iodine (I^{131}) and urinary excretion of radioactive iodine were measured. The rats were kept on the diets for seven weeks, after which the metabolic studies were performed. Also the groundnut was analyzed for the presence of known goitrogenic substances.

After seven weeks most of the groups receiving groundnut fractions had enlarged thyroid glands, showed increased percentages of inorganic iodine in the thyroid glands, and showed increased excretion of administered isotopic iodine. Of particular interest was the observation that a water extract of the groundnut cake exerted as great an effect as 20 parts of groundnut cake included in the basal diet, whereas groundnut protein was without effect. One microgram of potassium iodide prevented the changes induced by feeding 20 parts of groundnut cake.

Upon obtaining this evidence of goitrogenic action, the authors analyzed groundnut cake for various known goitrogenic agents. It was found to be free of thiooxazolidones and cyanogenetic glycosides. It was not high in calcium nor was their evidence of an accumulation of para-aminobenzoic acid.

Subsequently it was observed that groundnut meal containing a larger amount of the red pigmented skin caused a greater enlargement of the thyroid gland in albino rats than did diets containing depigmented meal. Therefore the authors investigated the effect of the glycoside found in the red skin of the groundnut.

The glycoside, arachidoside, was isolated from goundnut meal and was found to induce thyroid enlargement in albino rats when it was fed at a level of 10 mg. per rat per day. This effect was prevented by a concomitant supplement of 10 micrograms of potassium iodine per day. Urine analyses showed that there was a substantial increase in the excretion of free phenol, total phenol, glucuronide and ethereal sulfate after feeding the glycoside, but no unchanged glycoside could be detected. Further, the glycoside caused increased excretion of total iodine in the urine, the majority of which was found to be in the phenolic fraction. Histological examination of thyroid slices showed a typical picture of hypothyroidism in rats receiving the glycoside.

Experiments *in vitro* were conducted to determine the effect of incubating the glycoside with iodine (in potassium iodide solution) or with bromine water at pH 5.8 in phosphate buffer at 37°C. for 24 hours. In both cases precipitates formed which could be shown to contain a high percentage of iodine or bromide after being washed with ether and dried.

The results of both the *in vivo* and the *in vitro* experiments are taken as evidence suggestive of the presence of an "aromatic thyroid inhibitor" (Fawcett and Kirkwood, *J. Biol. Chem.* **204,** *787 (1953)*) in the groundnut. It appears probable that phenolic metabolites of the glycoside, arachidoside, are preferentially iodinated leading to a depletion of iodine for thyroid hormone synthesis. Even if future studies lead to a more complex explanation of the mechanism of action of this glycoside than the very suggestive evidence presented by the authors would suggest, there can be no doubt that one more naturally occurring goitrogenic agent has been identified as a result of this methodical study.

ELUCIDATION OF BACTERIAL GROWTH FACTORS

Working with meiotrophic mutants (mutants requiring fewer growth factors than the prototype) of *Pasteurella pestis*, E. Englesberg and L. Ingraham (*Proc. Nat. Acad. Sci.* **43**, *369 (1957)*) have developed a radically new method for the elucidation of the amino acid requirements of bacteria.

P. pestis A1122b1 has been previously shown to require cysteine, phenylalanine, methionine, valine, and isoleucine for growth at 30°C. in a mineral glucose medium. These requirements seem to be those of the wild type. Examination of the cysteine, methionine requirement has demonstrated just two breaks in the biosynthesis of these amino acids, one in the conversion of sulfate to sulfite and the other in the conversion of cysteine to cystathionine.

A methionine-independent mutant was isolated from the original wild colony by streaking [illegible] thiosulfate, valine, isoleucine and p[illegible]nine supplemented agar medium. [illegible] the isolation of this mutant, referred [illegible]$^+$, the possibility of isolating mutants independent of the other amino acids was explored. Since valine and isoleucine are both required as a result of a loss of a common enzyme in their biosynthesis, a mutant was readily isolated by streaking on a medium containing only the amino acids cysteine, phenylalanine, and methionine. This suggested that by a two-step mutation and selection procedure, a strain could be developed which would grow in a medium containing only cysteine and phenylalanine.

When the valine-independent, isoleucine-independent mutant was streaked heavily onto agar containing only cysteine and phenylalanine, it failed to grow and secondary mutant colonies were isolated which were found to be methionine-, valine- and isoleucine-independent, apparently confirming the original supposition. However, an entirely different picture developed when the mutants were selected in the reverse order. When the *me*$^+$ meiotroph isolated on a medium containing cysteine, phenylalanine, valine, and isoleucine was streaked onto a cysteine-, phenylalanine-containing agar, growth of the entire population occurred. This suggests that methionine had been acting in some manner as an inhibitor of valine and isoleucine synthesis and the valine, isoleucine "requirement" of the true prototype is actually to overcome inhibition. Thus the addition of valine and isoleucine to media containing cysteine, phenylalanine and methionine "neutralizes" the methionine inhibition and allows for growth of the prototype.

Subsequently, mutants not requiring methionine, valine or isoleucine were isolated directly from the prototype on cysteine, phenylalanine media and were found to be similar, if not identical, to those mutants isolated on agar containing cysteine, phenylalanine, valine and isoleucine. That this was a single mutational event was demonstrated by the finding that the viable counts on cysteine plus phenylalanine and cysteine, phenylalanine, valine and isoleucine agar plates of portions of the secondary colonies isolated from the prototype on cysteine, phenylalanine and cysteine, phenylalanine, valine and isoleucine were the same and were equal to that on a cysteine, phenylalanine, methionine, valine and isoleucine medium. Thus the valine, isoleucine, methionine "requirements" seemed to be lost simultaneously.

In further experiments isoleucine and valine were found to be inhibitory to growth when each was present alone, but when present together they apparently "neutralized" the individual inhibitory effects. In fact it appears that isoleucine is required to "neutralize" the methionine inhibition, and is in itself inhibitory, while valine is required to "neutralize" the isoleucine inhibition.

The value of the above mutational procedures is evident when it is realized that the "requirement" for valine and isoleucine, as involved in the "neutralization" of the methionine inhibition, could not have been discovered without first isolating a methio-

nine-independent mutant. Thus it seems that this approach is essential for the unambiguous explanation of growth-factor requirements of other commonly employed micro-organisms such as Lactobacillus, Streptococcus, Clostridium, and others. Perhaps some of the essential amino acids for human nutrition may actually function in a manner quite differently from that indicated by the type of evidence available at present.

BIOLOGICAL VALUE OF ALGAE PROTEIN

The high yield of food potentially available from cultured algae has made studies of this source of utmost importance in a world in which presently available sources of food may not keep pace indefinitely with the population increase. Moreover, the ease with which the yields of protein, fat, and carbohydrate can be increased or changed relatively by slight manipulation of the culture conditions makes possible the production of a great variety of foods from this single source (*Nutrition Reviews* **9**, *347 (1951)*; **11**, *77 (1953)*). It is particularly vital that a potentially high protein food of this type be made available to those areas in which large portions of the population exist in a state of protein malnutrition, because of lack of animal protein or because low-protein vegetables form a major part of the diet.

Yet, very few studies have been conducted on the value of algae protein as a major part of animal diets, although the few which have been reported have indicated that up to 20 per cent of dried chlorella in the diet was beneficial for chicks and acceptable to human beings (*Nutrition Reviews* **13**, *13, 45 (1955)*).

Over several years, H. Fink and his coworkers have carried out experiments designed to test the biological value of protein from unicellular organisms. In a recent study, Fink and E. Herald (*Z. f. physiol. Chem.* **305**, *182 (1956)*) tested the quality of the protein of green algae as compared with milk protein. The algae (*Scenedesmus obliquus*) was dried under an infra-red lamp and finely powdered. Two types of diets were tested, one in which the protein-equivalence was calculated on the basis of gross protein (N × 6.25) and the other in which it was calculated on the basis of "digestible" protein. In both diets, the principal protein (algae or milk) amounted to about 20 per cent, the remainder being made up of rye and wheat bread, yeast, cod-liver oil, salt mixture and starch. The protein itself consisted of 77 per cent algae protein, 15 per cent cereal protein and 8 per cent yeast protein.

Groups of 6 to 20 weanling rats were raised on these diets for 120 days and their weight gain was compared. In the first study, rats on the algae and milk diets showed similar satisfactory gains, far superior to diets which had been reported in a previous study in which the protein sources were from a variety of heterotrophic microorganisms (H. Fink and A. Hock, *Z. Naturforsch.* **2b**, *187 (1947)*). In the second experiment, the rats on the algae diet grew significantly faster than did those on the milk powder diet. At every stage, the rate of growth per gram of protein was slightly greater for the algae diet than for the milk-powder diet (*e.g.* 2.78 g. compared with 2.43 g. at 40 days). However, this was not the complete story, since the rats on the algae diet consumed more food and thus gained proportionally more than would be indicated by the relative values per gram.

Three animals on the milk powder diet died between the fortieth and forty-fifth days with signs of liver necrosis. This condition was not found in the animals on the algae diet on autopsy after the 120-day period.

From this study, it appears that not only

can algae serve as an efficient source of protein, but also that this protein has high biological value. Fink and Herald (*loc. cit.*) state that objections can be raised to the present experiments because the algae protein was not the sole source of protein. Experiments in which the cereal and yeast protein are omitted are soon to be reported.

HEME SYNTHESIS IN PANTOTHENIC ACID AND PYRIDOXINE DEFICIENCIES

Anemia frequently occurs in animals deficient in pantothenic acid or pyridoxine (*Nutrition Reviews* **8,** *15, 156 (1950)*). The nature of the anemia encountered in these diseases suggests that hemoglobin synthesis is impaired. Recent work has elucidated many of the steps in the biosynthesis of the prosthetic group of hemoglobin. Glycine and succinate are combined to form delta aminolevulinic acid, which is converted through intermediates to heme. Since succinate utilization in biochemical reactions requires coenzyme A to form succinyl CoA, it would be logical to expect impaired heme synthesis in pantothenic acid deficiency. The microcytic nature of the anemia in pyridoxine deficiency similarly could be the result of a block in heme synthesis.

The influence of pyridoxine and pantothenic acid deficiencies on heme synthesis has been directly tested by M. P. Schulman and D. A. Richert (*J. Biol. Chem.* **226,** *181 (1957)*). Carbon-14-labeled glycine or succinate was incubated with blood from normal and deficient ducks. Heme was subsequently isolated from the red cells and its radioactivity was determined.

In the first experiments, the effects of pyridoxine deficiency were studied. It was found that a deficiency of this vitamin resulted in a decreased incorporation of glycine into heme, but was without effect on the incorporation of aminolevulinic acid into heme. The *in vitro* addition of pyridoxal phosphate, the coenzyme form of pyridoxine, stimulated the incorporation of glycine and succinate into heme by deficient duck red blood cells, but again aminolevulinic acid utilization was not affected. Thus the results suggest that pyridoxine is required for the formation of aminolevulinic acid from glycine and succinate.

Pantothenic acid deficiency affected heme synthesis in a similar manner. Blood cells from ducks deficient in this vitamin utilized glycine and succinate for heme synthesis at a reduced rate, but the utilization of aminolevulinic acid was unaffected. The *in vitro* addition of calcium pantothenate or coenzyme A to the deficient duck blood did not restore heme synthesis to normal. However, the injection of pantothenic acid into the ducks one or two hours before they were killed was effective.

The results of these experiments permit a rational explanation for the anemia which accompanies deficiencies of pantothenic acid and pyridoxine. The two vitamins function in the synthesis of aminolevulinic acid, a precursor of heme which is the prosthetic group of hemoglobin.

THE BIOLOGICAL ROLE OF ALUMINUM

Since aluminum is one of the most widely used metals and is second only to silica as the most abundant constituent of the earth's crust, its effect upon man has been the subject of considerable study. I. R. Campbell, J. S. Cass, J. Cholak and R. A. Kehoe (*Arch. Ind. Health* **15,** *359 (1957)*) review in detail most of the available data about aluminum's physical and biological properties, uses, and effects.

The aluminum content of soil may range as high as 37.1 per cent, averaging about 15.3 per cent in the earth's crust. Aluminum is also found in the atmosphere (*i.e.* 2.5 to 6.0 gamma per cubic meter in Los Angeles). It may be quite concentrated in areas where much coal is burned, since the ash of coal contains 5.3 to 52.3 per cent aluminum. The aluminum content of vegetation averages 0.02 per cent of dry weight in the leaves and 0.002 per cent in the wood. Some plants are "accumulators". One species had 40.9 per cent aluminum content of wood, dry weight. Whether aluminum is essential for the growth of plants is not yet known. It has been thought to have some role in the uptake of water by plants, to be necessary for the best development of certain plants, (*i.e.* corn, rhododendron, blueberries and ferns), and to be necessary for the setting of seed.

The concentration of aluminum found in most fresh water is negligible, since the compounds that occur in water are soon precipitated or adsorbed upon sediment. The highest contents of aluminum are in the waters of volcanic districts. One thermal spring had a content of 0.09 per cent. At the other extreme, the aluminum concentration of a creek basin in Ohio ranged from 0.5 p.p.m. to 29 p.p.m., increasing with increasing acidity. Aluminum sulfate as a coagulant in water purification has been used since 1885, being currently added to water at the rate of 5 to 40 to 70 p.p.m. However, the resultant concentration of aluminum is less than it is in raw water. It averaged 21 p.p.m. in the untreated water of the Ohio river and 0.04 p.p.m. in the treated water.

Certain aluminum compounds have been added to foods. Alum (hydrated aluminum sulfate) is used to preserve the firmness of pickles, maraschino cherries, and frozen strawberries. Sodium aluminum sulfate has been widely used in baking powders as a substitute for cream of tartar, to liberate carbon dioxide from sodium bicarbonate. Using two heaping teaspoons (14 g.) of baking powder per cup of flour, the aluminum content of a finished biscuit would be about 0.4 g. aluminum per 100 g. The concentration of aluminum in foods after cooking in aluminum utensils fell within the range of concentrations in raw products, except for a few isolated instances, where the excess is not of physiological significance. The use of aluminum containers in the processing and packaging of foods causes no additional destruction of nutrients, discoloration, or flavor change beyond that caused by the process itself.

The amount of aluminum in the average diet, including the amounts from aluminum utensils, ranges from less than 10 mg. to 100 mg. per day. The lethal dose for experimental animals of aluminum acetate is 5 to 15 g. per kilogram. Anhydrous sodium alum is lethal for the rabbit at a dose of 2 g. per kilogram. On the other hand, a mixture of insoluble aluminum phosphates (35 per cent normal phosphate and 65 per cent pyrophosphate) can be given to rabbits by stomach tube up to the limits of feasibility, without harmful effects. Since the aluminum content of the acetate salt is greater than that of the chloride salt, the toxicity, which is in inverse proportion, evidently depends to a considerable extent upon the nature of the acid radical. Experiments with up to 1 g. of aluminum per day orally in humans, 8 mg. per kilogram in rats and 529 mg. per day in dogs, yielded no evidence of absorption. Human intake of greater than 200 mg. of aluminum per day binds a certain amount of phosphate. However, as much as 1118 mg. per day as aluminum hydroxide and 970 mg. per day as aluminum chloride do not produce phosphorus deficiency although phosphorus may be deflected from the urine to the feces with the phosphorus balance unchanged. Thus, a daily intake of 240 ml. of aluminum hydroxide may change the route of phosphorus elimination to the feces. The dietary addition of aluminum in concentrations of 1400 p.p.m. on the other hand, was found to lower markedly the inorganic phosphorus level in the blood and bones of guinea pigs,

rabbits and baby chicks. This amount is sufficient to combine with the total phosphorus.

After the administration of medicinal amounts of aluminum (10 to 100 times the normal dietary content), gastric pepsin is inactivated (but not destroyed) in varying degrees depending upon the concentration of aluminum. Acidity falls without affecting the acid-base balance. Gastric secretion is decreased or unchanged, and the pancreatic juice is unaltered. The constipating effect of aluminum has been attributed to its astringent action. The injection of aluminum results in its localization in various tissues and organs. The aluminum content of the livers and spleens of injected guinea pigs was elevated for at least one year afterwards. The lethal subcutaneous dose of aluminum for rabbits is 100 mg. per kilogram. Concentrations of aluminum greater than 1 per cent in intravenously injected solutions resulted in precipitation within the blood stream and subsequent embolization.

The effects of inhaled aluminum are controversial. Some workers claim the existence of a syndrome of pulmonary irritation and fibrosis. However, for over ten years, a standard treatment for silicosis utilized the inhalation of powdered aluminum, aluminum oxide, or aluminum hydrate, without a documented instance of an adverse tissue reaction. Other constituents of irritant dusts may be responsible for the pulmonary syndromes reported due to aluminum. The issue remains in doubt.

Judging from the urinary excretion of aluminum, the total amount absorbed per day is in the range of 100 gamma. The levels of concentration in tissue range from 0.004 to 0.5 mg. per 100 g., but the data are meager. The estimated total body content of aluminum is from 50 to 150 mg. Apparently, virtually all of the ingested aluminum is excreted in the feces, even on dietary amounts up to 300 mg. and more daily. Elevated contents of aluminum have not been demonstrated in bile, lymph or other secretions after ingestion.

In summary, the absorption of aluminum is so slight as to escape detection except by tissue analysis after long exposure, and is relatively independent of mass effect. The physiologic role of aluminum is obscure.

DIET AND GALLSTONES

According to C. J. Watson (*A Textbook of Medicine, ed. R. L. Cecil and R. F. Loeb, W. B. Saunders Co. Phila. (1955) pp. 946–955*), gallstones occur in about 5 to 10 per cent of all adults over 40 years of age. The incidence is considerably higher among women, especially among those who have borne one or more children. The presence of gallstones is associated with an increased level of cholesterol in the blood and in the bile. The hypercholesterolemia occurs late in pregnancy and for a time after delivery. It may be that increased cholesterol metabolism and biliary stasis in pregnancy may partially account for the increased incidence of gallstones in women. However, hypercholesterolemia alone is not enough to produce gallstones, since there are some conditions (*e.g.* myxedema and nephrosis) where the high levels of cholesterol are not associated with the formation of stones.

Gallstones, according to J. A. Kolmer (*Clinical Diagnosis by Laboratory Examination, Appleton-Century Crafts, Inc. New York (1949) p. 787*), can be classified as (1) mixed or mulberry stones, which are the commonest ones occurring in man and are composed largely of cholesterol along with some calcium carbonate and traces of iron, manganese, copper and other substances; (2) cholesterol stones, in which the sterol is present to the extent of 99 per cent; (3) pigment stones, which contain primarily bilirubin combined with calcium; and (4)

calcium stones, containing almost nothing but calcium phosphate.

It has been suggested by A. R. P. Walker (*Nutrition Reviews* **14,** *321* (*1956*)) that formation of gallstones may be influenced to a certain extent by dietary factors. He points out that from a series of approximately 9000 consecutive autopsies in South Africa, the incidence of gallstones was 2 per cent among the Bantus and 13.5 per cent among the local Europeans. Obviously there are other differences between these two groups, besides diet, which may be important in the causation of gallstones.

Another suggestion for the dietary origin of gallstones comes from R. M. Kark (*Modern Nutrition in Health and Disease, ed. M. G. Wohl and R. S. Goodhart, Lea and Febiger, Phila.* (*1955*) *p. 622*). He states that gallstones are rarely seen among the Japanese, but that they are common among the peoples who live on a high calorie, high fat diet. He notes that: "While there is no proof at present that dietary habits are the prime cause of cholecystitis (inflammation of the walls of the gall bladder and bile duct) and gallstones, the data collected thus far is (sic) highly suggestive."

Prior to 1950 there were only a few reports in the literature on the development of gallstones in experimental animals. One of these was by R. Okey (*Proc. Soc. Exp. Biol. Med.* **51,** *349* (*1942*)). She noticed that when guinea pigs were fed a diet containing in per cent casein 20, crisco 15, wheat bran 5, brewers' yeast 10, salts 2.5, agar 2.5, cornstarch 44, and cholesterol 1, they showed cholecystitis and, in some cases, the bile ducts were impacted with what appeared to be gallstones. These stones contained primarily calcium phosphate, although some cholesterol was present in them. They did not appear in the animals raised on the same diet free of cholesterol.

Over the past six years, H. Dam and F. Christensen have reported on the occurrence of gallstones in hamsters raised on diets free of cholesterol and very low in fat. These studies were initiated in an attempt to produce muscular dystrophy in hamsters. The occurrence of gallstones was so intriguing that the experiment was changed to study the factors involved in the development of gallstones (*Acta Path. et Microbiol. Scand.* **30,** *236* (*1952*)).

Weanling hamsters were placed on a fat-free diet containing in per cent casein 20, sucrose 74.3, salt mixture (no. 2) 5, and all of the vitamins required by the hamsters in adequate amounts. The fat-soluble vitamins were given as a stabilized aqueous suspension. The animals grew very slowly on this diet and developed skin lesions which were more severe than those reported as occurring in rats on a fat-free diet. Ulcerations occurred at the junction of the mucosa and epidermis; this was especially severe around the anus. The kidneys were pale or showed pale spots on the surface. Sixteen of the 18 animals killed after 87 days on the diet showed gallstones. However, only one out of 14 animals killed earlier than this showed any stones.

The livers of the animals which had gallstones showed a marked mosaic structure on the surface. The bile in the gall bladders of the animals with stones appeared normal. The stones could be seen through the gall bladder; they were yellow, brown, green, or almost white. The size of the stones varied, with some of them being small enough so that they could probably pass through the bile duct without much difficulty. The concentration of cholesterol in the stones ranged from 50 to 94 per cent, with the large part of the chloroform-insoluble fraction composed of proteins which presumably formed the matrix of the stones.

Christensen, Dam and I. Prange (*Acta physiol. Scandinav.* **27,** *315* (*1952*)) found that the gallstones appearing in the hamsters were not related to the fat deficiency syndrome produced by the diet. Feeding the hamsters linoleic acid, or adding 10 per cent lard to the diet, prevented the skin symptoms but had no effect on the formation of

gallstones. Six per cent alfalfa meal replacing the same amount of sucrose in the diet had no effect on the formation of gallstones. The substitution of ground whole wheat or ground rolled oats for all of the sucrose (72 per cent) reduced the incidence of gallstones to 50 per cent, or less, of the animals. Ground durra (a variety of sorghum) protected all animals from gallstone formation.

In an extension of the above work, Christensen and Dam (*Ibid.* **31,** *75* (*1954*)) found that when soy beans and Fleischmann's yeast (type 2019) were added to the lithogenic diet (one tending to produce gallstones), they were very effective in preventing the formation of gallstones. Hempseeds, linseeds, rapeseeds, peanuts, yellow peas, white rice, wheat, rye and barley were less effective. The protective factor was not oil-soluble as shown by the activity of extracted hemp meal and the inactivity of soy bean oil; furthermore, it was not extracted from yeast by hot water.

Another purified diet, differing from the one used in the above studies only in the salt and vitamin mixtures, did not produce any stones when fed to hamsters (Christensen and Dam, *loc. cit.*). The diet producing the gallstones contained salt mixture No. 2, U. S. P. XIII, while the non-lithogenic diet contained salts No. 185 of McCollum and Simmond, supplemented with copper and manganese sulfates and potassium iodide. The addition of copper sulfate to the lithogenic diet at levels of 0.04, 0.13 and 0.40 per cent produced a variable protective effect which was not consistent from trial to trial.

The above workers (*Ibid.* **27,** *315* (*1952*)) also found that the hamsters developing gallstones had the same blood cholesterol level and ratio of esterified and free cholesterol as those on the diet containing durra.

Since a large portion of the gallstones in the hamsters contained cholesterol, H. K. Hanel, Christensen, and Dam (*Acta Path. et Microbiol. Scand.* **35,** *237*, *423* (*1954*)) studied the rate of cholesterol synthesis by the liver of hamsters fed the diet previously described. After weanling hamsters had been on the diet for two months, they were injected with carbon-labeled acetate. Twenty-four hours after injection, the animals were killed and the livers removed. The radioactivity of the isolated cholesterol was the same, regardless of whether the animals had or had not gallstones.

Not only could gallstones be produced in hamsters by dietary means, but once they had been formed they could be reduced in size by a change in diet. Christensen, Dam and G. Kristensen (*Acta physiol. Scandinav.* **36,** *329* (*1956*)) fed a group of hamsters the low fat diet (the above lithogenic diet containing 2 per cent lard) which produced gallstones in all ten animals. Weanling animals were maintained on the diet for 63 days. Another group was maintained on the above diet for an additional 77 days. The third group was maintained on it for 63 days, and then transferred to a diet containing in per cent casein 20, Fleischmann's yeast (2019) 36, polished rice 28, salts 5, vitamin mixture 0.7, and lard 10. All but one of the animals maintained on the lithogenic diet for 140 days had gallstones. Of the group that was transferred to the yeast and polished rice diet, only five of the ten animals had stones. The cholesterol content of the stones in the animals maintained on the lithogenic diet throughout life was 55 per cent. The stones in the animals transferred to the yeast-rice diet contained less than 0.7 per cent cholesterol. The latter stones were hard, amorphous, and dark colored.

Not only did the basal diet produce gallstones in the hamsters, but it also resulted in depigmentation of the incisors. The golden-brown color of the normal incisors returned when the hamsters were shifted to the yeast-rice diet. The presence of the gallstones was also associated with a pale grey fur, whereas the animals receiving the yeast-rice diet had a normal red-brown fur. The shift in the diet was associated with a prompt

increase in body weight in both the males and females. Nothing was done to determine whether vitamin E was associated with the changes in the incisor. Earlier work of Dam and co-workers (H. Granados, K. E. Mason and Dam, *J. Dent. Res.* **24,** *197 (1945)*) showed depigmentation of the incisors in hamsters to be a symptom of vitamin E deficiency.

The above work is significant in that it indicates gallstones can be formed and dissolved by dietary manipulations. The intriguing point about the work is the suggestion of the evidence that causative and protective factors are associated with foods not primarily lipid in nature. The hamsters developed gallstones on diets free not only of cholesterol but also of fats, and were cured by such foods as yeast, soy beans, and a number of other seeds. However, the protective factor was not present in the oil.

At present, it is difficult to determine the exact factor(s) responsible for the formation of the gallstones observed by Dam and coworkers. Stones appeared only in the hamsters fed a diet differing from a non-lithogenic diet solely in the salts and vitamins. Undoubtedly, salts (perhaps others besides copper) play some role in protecting the hamsters from gallstones. It is equally tenable that other salts may precipitate their formation.

The primary drawback to the above work is that the weanling hamsters barely gained weight at all during the 12 weeks required for the development of the gallstones. The starting weights of most of the animals used in these experiments were between 40 and 45 g. By the time the gallstones were evident, the animals weighed between 60 and 66 g. This is approximately $\frac{2}{3}$ the weight of the normal adult hamster. Although no mention is made of biliary disturbances during starvation (A. Keys *et al. The Biology of Human Starvation, Univ. Minn. Press, Minneapolis (1950)*, **Vol. 2**), it would be advisable to restrict the weight gain of the animals receiving one of the potent supplements in order to make sure that the stones are not a manifestation of retarded development. The relation between poor weight gain and gallstone formation should be clarified, since stones, occurring primarily in adults, are seen infrequently in young people.

Gallstones have also been observed in rabbits fed a commercial stock diet supplemented with dihydrocholesterol (M. Bevans and E. H. Mosbach, *A. M. A. Arch. Path.* **62,** *112 (1956)*). None of the 18 rabbits (starting weight 1.8 to 4.2 kg.) maintained on the stock ration developed any stones. All 25 animals fed the diet containing dihydrocholesterol at levels of 0.25, 0.5 or 1.0 per cent showed gallstones after as little as one week. Rabbits fed the dihydrocholesterol for three to six weeks had gall bladders that were filled with small stones. The walls of the gall bladders of these rabbits were slightly edematous. Addition of 12 per cent olive oil to the diet containing dihydrocholesterol produced no change in either the incidence of gallstones or the pathologic changes in the gall bladder. Neither 1 per cent cholesterol nor 1 per cent β-sitosterol added to the stock ration, with or without the 12 per cent olive oil, produced any gallstones in the rabbits.

The gallstones contained less than 0.02 per cent of digitonin-precipitable sterols (cholesterol, sitosterol, dihydrocholesterol, etc.) but did contain large amounts of deoxycholic and glycocholic acids. These acids are normal constituents of rabbit bile.

Bevans and Mosbach (*loc. cit.*) suggest that the dihydrocholesterol in the diet of rabbits produces an inflammation of the walls of the gall bladder, and also renders the bile acids less soluble. Under these circumstances, the bile acids precipitate and form stones.

It has been shown that dihydrocholesterol in the diet of hens reduces the blood cholesterol level (*Nutrition Reviews* **12,** *54 (1954)*).

However, dihydrocholesterol holds no promise of being therapeutically valuable in treating hypercholesterolemia, since the compound is absorbed and appears both in the blood and the atheromatous plaques of rats and rabbits (C. W. Nichols, Jr., S. Lindsay and I. L. Chaikoff, *Proc. Soc. Exp. Biol. Med.* **89,** *609 (1955)*; G. L. Curran and R. L. Costello *Ibid.* **91,** *52 (1956)*).

The above work should spur clinical investigators to study, for their effect on gallstones, some of the dietary components known to reduce the cholesterol content of the blood. Suggestive evidence that such an effort may be worthwhile comes from an observation by M. C. Miller (*Gastroenterology* **31,** *588 (1956)*) of the spontaneous disappearance of gallstones in a young woman. The stones developed during her pregnancy and disappeared two months after delivery.

The experimental studies add further suggestive evidence for the postulate that diet plays some role in the development of gallstones, and they offer a hope that proper dietary procedures may be of value in dissolving stones already formed.

GLYCINE METABOLISM IN TISSUES

That the amounts of labeled amino acids incorporated into proteins vary greatly with different tissues was observed by R. Shoenheimer, S. Ratner, and D. Rittenberg (*J. Biol. Chem.* **130,** *703 (1939)*). This work was performed on rats using L-leucine which had been labeled with both N^{15} and deuterium. Later work, in which carbon- and sulfur-labeled amino acids were used, has confirmed the observations that the mixed proteins of some tissues such as intestinal tract, liver, and kidney contain large amounts of newly supplied amino acids within a few hours after injection, while other tissues such as muscle, skin and brain give only low specific activities. Schoenheimer and Rittenberg (*Physiol. Rev.* **20,** *218 (1940)*) concluded that the concentration of isotope found in a particular tissue under the conditions described could be taken as a measure of "relative chemical activity" of the organ proteins.

With tissues, such as liver and kidney, containing proteins which are being replaced at rapid rates, maximum radioactivity is reported as being reached within about one hour after administration of the labeled compound (H. Borsook *et al., J. Biol. Chem.* **187,** *839 (1950)*). The specific activity remained constant for several hours and then decreased to reach half-maximum values in periods ranging from three to ten days, depending on the species and the tissue. O. B. Henriques, S. B. Henriques and A. Neuberger (*Biochem. J.* **60,** *409 (1955)*) have pointed out that it should be possible to calculate turnover rates of proteins from the ascending limb of the activity time curve of the protein, provided that the value of specific activity of the free amino acid during the relative periods is known.

Since such data were not available, such workers as D. Shemin and Rittenberg (*J. Biol. Chem.* **153,** *401 (1944)*) were forced to base their estimate of the half-life value of the average protein molecule in the rat liver on the assumption that the N^{15} content of the free glycine available for protein synthesis some time after administration was equal to that of the glycine obtained on hydrolysis of the carcass protein. In the early work on the turnover of plasma proteins, labeled amino acids were used and it was tacitly assumed that the content of the amino acid "pool" had dropped to negligible values within 12 to 24 hours after administration. When labeled proteins are transferred, the error arising from neutralization of the labeled amino acid liberated on breakdown of the injected protein is likely to be much smaller than when a labeled amino

acid is used. However, Henriques *et al.* (*loc. cit.*) point out that even in these latter experiments it would be desirable to know the specific activity of the free amino acid compared with that of the protein-bound amino acid measured at the same time.

In view of the questionable status of this field of study, Henriques *et al.* (*loc. cit.*) recently undertook the task of isolating free and protein bound glycine from blood, liver, and muscle at various times after administration of labeled amino acid and of estimating its radioactivity. Glutathione from liver and muscle was also isolated and the radioactivity of the glycine obtained by its hydrolysis was measured. In addition, glycine was obtained from the hydrolysates of the mixed proteins of liver and muscle.

Twenty-two female albino rabbits, each weighing about 2 kg., were used. After being fasted for three hours they received 5 microcuries of C^{14} glycine intravenously. Blood was taken from the aortas, and whole livers and the muscles of both hind legs were removed 5, 10, 15, 30 minutes and 1, 2, 3, 4, 6, 12 and 18 hours respectively, after the injection of glycine. The animals killed at 12 and 18 hours received 25 microcuries of C^{14} glycine. Blood serum, muscle or liver powder were treated with equal weights of ten per cent trichloracetic acid (TCA). Glutathione was precipitated as its cuprous mercaptide. Washed proteins were ground to a fine powder. Glycine for counting was isolated chromatographically from TCA extracts and hydrolyzed proteins by treatment with 1-fluoro-2:4 dinitrobenzene which yielded 2:4 dinitrophenyl glycine.

It was found that the radioactivity of the glycine in the plasma decreased sharply during the first 30 minutes, less rapidly in the next few hours and very slowly during the period extending from six to 18 hours after the injection of the labeled amino acid. The authors interpret these results as indicating that dilution of the labeled plasma glycine occurred by several reactions with widely different rates.

The exchange of plasma glycine with free liver glycine was calculated to be complete between 15 and 20 minutes. It was concluded that permeability barriers play only an insignificant part in this exchange, and that the limiting factor is the rate of blood flow. Exchange between plasma glycine and free muscle glycine was very much lower and the time for complete free amino acid exchange was estimated to be about 20 to 25 hours. The authors concluded that this slow equilibration is caused by both a low rate of blood flow in muscle and the existence of a permeability barrier.

There occurred a rapid reversible conversion of glycine into serine. The specific activity of muscle serine was appreciably higher than that of muscle glycine, suggesting that the muscle cell is more permeable to serine than to glycine.

Exchange times of glutathione were calculated to be about 10 to 12 hours in the liver and 20 hours in muscle. By making various assumptions, it was calculated that approximately 1.9 g. of protein are synthesized in the liver per day per kilogram rabbit. About 40 per cent of the total synthesis seems to be concerned with plasma proteins and 60 per cent with liver proteins proper. Although the low incorporation of labeled glycine into muscle proteins is caused in part by the low rate of penetration of glycine into muscle, the authors concluded that the rate of protein synthesis in muscle is also much smaller than that in liver, although no quantitative calculations could be made.

As turnover rates and conversions of various amino acids become known in increasingly more detail, the nutritionist will eventually be in possession of a rather sound basis with regard to prescribing diets for specific nutritional situations. The information that muscle cells are more permeable to serine than to glycine immediately suggests investigation along several practical applications of such a phenomenon. Increasing the efficiency of new protein formation by various combinations of amino acids is

another problem which is most satisfactorily attacked when armed with foreknowledge regarding the actions of the specific amino acids in a tissue environment.

NOTES

Letters to the Editor

Sir:

We are pleased that two of our articles were reviewed in the May 1957 issue of *Nutrition Reviews* (page 143). We believe, however, that we should point out the existence of three errors in the review.

In paragraph 2, line 18, the reviewer misquotes us when he writes "when the contents are passed through the intestine and colon rapidly less *rapid* absorption occurs". Nowhere in the article did we say anything about the rapidity of absorption. In paragraph 3, line 7, the reviewer points out that "when 100 mg. of cholesterol per kilogram of body weight per day was supplied with these diets the values for apparent cholesterol absorption . . . were 73.7 and 81.8 milligrams per kilogram of body weight per day (*not per cent as the authors report*)". This is incorrect because our results were expressed as amount (mg.) as well as percentage absorbed. Since 100 mg. per kg. per day was fed, the figure for the apparent absorption is the same whether it is expressed in amount (mg.) or in per cent. This information about the intake and excretion was given in the same table. In paragraph 3, line 21, the reviewer states that "the conclusion that cholesterol absorption was less rapid would not appear to be justified." We drew no such conclusion. On the contrary, we concluded that the "absorption of dietary cholesterol was not reduced."

T. M. Lin
Esko Karvinen
A. C. Ivy
Department of Clinical Science
University of Illinois Chicago

Sir:

The Special Article by Professor D. M. Hegsted on "Calcium Requirements" (*Nutrition Reviews* **15,** *257 (1957)*) makes welcome reading to those of us living side by side with indigenous populations habituated to a low intake of calcium, yet without demonstrating stigmata. Indeed, as Professor Hegsted points out, "the signs and symptoms of a dietary calcium deficiency syndrome remain undescribed." If this is valid, is not the continual advocation of luxus intakes of a nutrient to protect against unspecified stigmata a highly questionable procedure? Surely there has been ample time for protagonists of high calcium intakes to adduce unequivocal evidence of the deleterious effects of sustained low calcium intakes among the poorer sections of populations. Failure in this respect cannot but lead to the entertainment of serious doubts of the legitimacy of calcium even to remain included in Tables of Recommended Allowances. Is it not very probable that throughout the world, every-day diets which support and maintain life contain enough of this important element to permit integrity of bone formation and the other physiological functions in which calcium is implicated?

A. R. P. Walker
Human Biochemical Unit
South African Institute of Medical Research
Johannesburg, South Africa

Sir:

In the review on the assay of unidentified chick growth factors in the March 1957 issue of *Nutrition Reviews*, you draw attention to the need for improved procedures

and discuss the composition of the basal diets used in assays.

We have been concerned with these problems and have been struck by the fact that, whereas workers in this field are careful to ensure that the basal diet contains generous amounts of all known vitamins and minerals, many workers overlook the fact that it may not contain optimum amounts of energy and protein. Some of the sources of unknown growth factors are very rich in fat and protein and, since they must be added to the diet in relatively large amounts in order to obtain a measurable growth response, the energy and protein content of the supplemented diets are often substantially different from the control. G. H. Arscott (*Poultry Sci.* **35,** *338 (1956)*), for example, replaced up to 12 per cent sucrose in his basal diet by dried egg yolk, which must have increased the protein content by approximately 3.5 per cent and the fat by 7 per cent, and J. H. Hopper, H. M. Scott and B. C. Johnson (*Ibid.* **35,** *195 (1956)*), replaced 20 per cent of the cerelose of their synthetic basal diet in one treatment by 5 per cent fresh egg yolk + 5 per cent liver powder + 5 per cent dried whey + 5 per cent fish solubles, thus producing a diet which bore little resemblance to the control ration with which it was compared.

In order that the observed growth response may be attributed with certainty to unidentified growth factors present in crude supplements, it seems to us essential to ensure that the basal diet used in assays for these factors does not support an increased rate of growth when supplemented with pure fat or protein, or fat and protein together.

Yours sincerely,
T. G. TAYLOR
J. H. MOORE
The University, Reading, England

Recent Nutrition Books

Annual Report of the National Institute of Nutrition (1956). The National Institute of Nutrition Toyamacho, Tokyo, Japan. Pp. 65.

Sterilization in Food Technology. C. Olin Ball and F. C. W. Olson. McGraw-Hill Book Co. Pp. 653. $16.00.

The Rowett Research Institute Collected Papers: Summary and Subject Reviews. Vol. XIII. Reid Library, The Rowett Research Institute, Bucksburn, Aberdeenshire. Pp. 47.

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